Glucocorticoid receptor gene haplotype structure and steroid therapy outcome in IBD patients

Mwinyi, J ; Wenger, C ; Eloranta, J J ; Kullak-Ublick, G A

Abstract: AIM: To study whether the glucocorticoid receptor (GR/NR3C1) gene haplotypes influence the steroid therapy outcome in inflammatory bowel disease (IBD). METHODS: We sequenced all coding exons and flanking intronic sequences of the NR3C1 gene in 181 IBD patients, determined the single nucleotide polymorphisms, and predicted the NR3C1 haplotypes. Furthermore, we investigated whether certain NR3C1 haplotypes are significantly associated with steroid therapy outcomes. RESULTS: We detected 13 NR3C1 variants, which led to the formation of 17 different haplotypes with a certainty of $> 95\%$ in 173 individuals. The three most commonly occurring haplotypes were included in the association analysis of the influence of haplotype on steroid therapy outcome or IBD activity. None of the NR3C1 haplotypes showed statistically significant association with glucocorticoid therapy success. CONCLUSION: NR3C1 haplotypes are not related to steroid therapy outcome.

DOI: https://doi.org/10.3748/wjg.v16.i31.3888

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v16.i31.3888

World J Gastroenterol 2010 August 21; 16(31): 3888-3896
ISSN 1007-9327 (print)

ORIGINAL ARTICLE

Glucocorticoid receptor gene haplotype structure and steroid therapy outcome in IBD patients

Jessica Mwinyi, Christa Wenger, Jyrki J Eloranta, Gerd A Kullak-Ublick

Jessica Mwinyi, Christa Wenger, Jyrki J Eloranta, Gerd A Kullak-Ublick, Division of Clinical Pharmacology and Toxicology, University Hospital, CH-8091 Zurich, Switzerland
Author contributions: Kullak-Ublick GA, Eloranta JJ and Wenger C designed the study; Wenger C and Eloranta JJ performed the laboratory experiments; Mwinyi J performed the sequence and statistical analysis; Mwinyi J, Eloranta JJ and Kullak-Ublick GA wrote the manuscript.
Supported by The Swiss IBD Cohort Study (SNF Grant 33CSC0-108792); the Swiss National Science Foundation (Grant 32-120463/1); the Zurich University Research Priority Programme "Integrative Human Physiology" (ZIHP); the Center of Clinical Research at the University Hospital Zurich; and the Novartis Foundation for Biomedical Research
Correspondence to: Gerd Kullak-Ublick, Professor, Division of Clinical Pharmacology and Toxicology, University Hospital, CH-8091 Zurich, Switzerland. gerd.kullak@usz.ch
Telephone: +41-44-2552068 Fax: +41-44-2554411
Received: March 11, 2010 Revised: May 14, 2010
Accepted: May 21, 2010
Published online: August 21, 2010

Abstract

AIM: To study whether the glucocorticoid receptor (*GR*/*NR3C1*) gene haplotypes influence the steroid therapy outcome in inflammatory bowel disease (IBD).

METHODS: We sequenced all coding exons and flanking intronic sequences of the *NR3C1* gene in 181 IBD patients, determined the single nucleotide polymorphisms, and predicted the *NR3C1* haplotypes. Furthermore, we investigated whether certain *NR3C1* haplotypes are significantly associated with steroid therapy outcomes.

RESULTS: We detected 13 *NR3C1* variants, which led to the formation of 17 different haplotypes with a certainty of > 95% in 173 individuals. The three most commonly occurring haplotypes were included in the association analysis of the influence of haplotype on steroid therapy outcome or IBD activity. None of the *NR3C1* haplotypes showed statistically significant association with glucocorticoid therapy success.

CONCLUSION: *NR3C1* haplotypes are not related to steroid therapy outcome.

Key words: Inflammatory bowel disease; Steroid therapy; Glucocorticoid receptor; Pharmacogenetics; Haplotype analysis

Peer reviewers: Uday C Ghoshal, MD, DNB, DM, FACG, Additional Professor, Department of Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Science, Lucknow 226014, India; Stephan Johannes Ott, PhD, MD, Clinic for Internal Medicine I, University-Hospital Schleswig-Holstein (UKS-H), Campus Kiel, Arnold-Heller-Str. 3, Hs. 6, 24105 Kiel, Germany; Ferenc Sipos, MD, PhD, Cell Analysis Laboratory, 2nd Department of Internal Medicine, Semmelweis University, Szentkirályi u. 46., Budapest 1088, Hungary; Hoon Jai Chun, MD, PhD, AGAF, Professor, Department of Internal Medicine, Institute of Digestive Disease and Nutrition, Korea University College of Medicine, 126-1, Anam-dong 5-ga, Seongbuk-gu, Seoul 136-705, South Korea

Mwinyi J, Wenger C, Eloranta JJ, Kullak-Ublick GA. Glucocorticoid receptor gene haplotype structure and steroid therapy outcome in IBD patients. *World J Gastroenterol* 2010; 16(31): 3888-3896 Available from: URL: http://www.wjgnet.com/1007-9327/full/v16/i31/3888.htm DOI: http://dx.doi.org/10.3748/wjg.v16.i31.3888

INTRODUCTION

Inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC), are multifactorial disorders, which are characterized by chronic recurrent inflammation of the gastrointestinal tract[1]. The molecular pathogenesis of IBD is not fully elucidated, although an exaggerated mucosal immune response triggered by intestinal bacteria

in genetically susceptible individuals appears to play an important role[2]. The combined prevalence of CD and UC is estimated to be 100 to 200 per 100 000 individuals in developed countries[3]. IBD shows extensive variation in individual clinical presentation and outcomes, which is likely to be caused by differences in genetic susceptibility, environmental factors, intestinal bacteria and activation of the intestinal immune system[3].

Although great advances have been made in the management and therapy of IBD, curative therapy does not yet exist. The anti-inflammatory agents mesalazine (5-aminosalicylic acid, 5-ASA) and sulfasalazine, in combination with glucocorticoids (GCs), are common first line therapy options in induction and maintenance of UC remission. Severe cases of UC are treated intravenously with GCs or cyclosporine. CD is mainly treated with GCs and/or antibiotics, and azathioprine (AZA), 6-mercaptopurine (6-MP), or the anti-folate methotrexate (MTX) are often added to maintain the state of remission[4]. GC-resistant or -dependent disease courses can be treated with anti-TNF-α antibodies, such as infliximab and adalimumab. GCs are often used in the initial treatment of most cases of moderate to severe active UC or CD. However, 20% of patients develop GC resistance within one year of treatment[5,6]. Non-response to GCs often leads to the need for a surgical intervention as a result of a poor therapy outcome. For example, it has been reported that 38% and 29% of steroid-resistant CD and UC patients, respectively, required surgery within one year after beginning GC treatment[5].

Glucocorticoids are potent inhibitors of T cell activation and cytokine secretion, primarily *via* binding to the cytoplasmically located glucocorticoid receptor (GR) as ligands. Due to ligand binding, homodimers consisting of two activated GRs are formed that translocate into the nucleus. The complex subsequently binds to specific glucocorticoid response elements (GREs) within the regulatory regions of GR target genes[7]. The mechanisms by which GC resistance develops, are not fully understood. Three possible mechanisms have been proposed. First, decreased plasma levels of GCs through overexpression of the drug efflux system P-glycoprotein (*MDR1*). Second, an altered function of GR or, third, excessive synthesis of pro-inflammatory cytokines induced by activation of pro-inflammatory transcription factors may reduce the affinity of GR to its ligands and lead to the development of GC resistance[4].

GR is known to be expressed as several polymorphic variants[8]. Several mutations in the *NR3C1* gene have been found to modulate individual GC sensitivity in *in vitro* investigations and in studies with healthy individuals[9,10]. In the present study we evaluated the association between the *NR3C1* gene haplotypes and therapeutic outcome of GC administration in a well-sized cohort of 181 patients with IBD. The aim was to comprehensively determine abundant GR variants by sequencing all protein-coding *NR3C1* exons (exons 2 to 9) and the first 50 bp of the neighbouring intronic regions in all individuals. We hypothesized that *NR3C1* gene polymorphisms may influence GC sensitivity and thus might serve as predictive markers for treatment success with GCs in IBD patients.

MATERIALS AND METHODS

Patients

One hundred and eighty-five clinically diagnosed Swiss IBD patients were recruited at the centers participating in the Swiss Inflammatory Bowel Disease Cohort Study (SIBDCS)[11]. All patients gave their informed consent for inclusion into the study. An ethical approval was obtained from the Medical Ethical Committees of the University Hospital Lausanne, Switzerland, and all local study sites. All patients had been treated with steroids and past steroid therapy outcome had been recorded. The standard employed criteria for the steroid therapy success or failure are available on the website www.epact.ch. Briefly, an insufficient response upon appropriate treatment in terms of doses and duration was considered a steroid therapy failure. EDTA-blood samples were stored at the central tissue repository at the Institute of Pathology, University of Bern, Switzerland. The SIBDCS data center at the University Hospital of Lausanne, Switzerland, provided data on past and current disease characteristics and GC therapy outcome. Diagnosis of IBD (CD or UC) was confirmed by the study investigators based on clinical presentation, endoscopic findings and histology.

Sequencing reactions

DNA was extracted from EDTA-blood using the QIAcube robotic workstation and a standard procedure (QIAamp DNA Mini Kit, QIAGEN, Switzerland). The PCR and sequencing primer design was based on the NCBI reference sequence (GenBank accession number NT_029289). Primers for genomic DNA were designed to span all expressed exons (2 to 9) and at least 50 bp of flanking intronic sequences at both 5'- and 3'-ends. The DNA sequences of purified PCR fragments were obtained with an ABI 3730xl sequencing machine. Details of the PCR primers can be found in the Table 1. Optimized PCR conditions, and methods used for subsequent purification and sequencing of the fragments are available upon request.

Haplotype analysis

The PHASE software was used to calculate the haplotypes based on the detected single nucleotide polymorphisms (SNPs) and mutations in the *NR3C1* gene. PHASE predicts *in silico* haplotypes on the basis of a Bayesian inference algorithm[12,13]. Haplotype calculations were performed on 181 individuals, from which sequence data of adequate quality were obtained. To allow referral to specific haplotypes, a frequency-based priority criterion was used to name them (e.g. *GR_1* for the most often occurring haplotype, Table 2).

Calculation of linkage disequilibria

Linkage disequilibria (LD) were calculated using the r^2 statistics. Calculations were performed using the software package Haploview (www.haploview.com).

Statistical analysis

To detect differences in haplotype distribution between

Table 1 Oligonucleotides used as polymerase chain reaction primers to amplify the *NR3C1* exons

Primer name	Primer sequence	Nested PCR	Primer name	Primer sequence
GR 2_F	CACTTAGGTTGTCTACCTTTCCTAC	Y	GR 2_Fa	TTCAAAAGGCCACTTAAACTTATTC
GR 2_R	GATAGAAACTACTCTTCTGGTAAC	Y	GR 2_Ra	CCTTGGAGATCAGACCTGTTG
		Y	GR 2_Fb	CTGTGCCCAGTTTCTCTTGC
		Y	GR 2_Rb	CAGCCAGATCTGTCCAAAGC
		Y	GR 2_Fc	TTGGAAACTCCTTCTCTGTGG
		Y	GR 2_Rc	AATGTGGCATGCTGAATGG
GR 3_F	CATTAGAGGACCTAGGAGCCAC	N		
GR 3_R	GAAGTGAACCAGAACCACACC	N		
GR 4_F	TGAATTCAGTGTGTGTAAGAAGAAC	N		
GR 4_R	TTGCACTGTTTTCAGTTTGTTG	N		
GR 5_F	CACCTGTATTCACCTGACTCTCC	N		
GR 5_R	TTTTTTCTCCTTTTCCATGTCAC	N		
GR 6_F	GCCCCAAGCACTCATAACTC	N		
GR 6_R	TCAGATGACAGAAGAAAACTGTGTC	N		
GR 7_F	AATCTTGGTGTCACTTACTGTGC	N		
GR 7_R	CCAAGATGCAGGAAGTTTAAGG	N		
GR 8_F	CACCAACATCCACAAACTGG	Y	GR 8_Fa	TTGGTCAGTGGGAACATC
GR 8_R	CCACCAGTTCTTCTTACACACAC	Y	GR 8_Ra	ATGGTGGCTTGTGCCTAC
GR 9a_F	TGATGACGACTCAACTGCTTC	N		
GR 9a_R	ATCTGGGGAATTCCAGTGAG	N		
GR 9b_F	TCCTAAAAGGGCACAGCTTC	N		
GR 9b_R	CAATCATTTGCTTTTTGAATGC	N		

PCR: Polymerase chain reaction; GR: Glucocorticoid receptor.

Table 2 Predicted haplotypes found to be in best reconstruction for 181 inflammatory bowel diseases patients

Haplotype number[1]	Haplotype composition[2] Reference: GGGATGGCCATGT	Absolute haplotype frequency (n = 362)	Relative haplotype frequency	Number of haplotypes not included[3]
GR_1	1111111111111	165	0.456	
GR_2[4]	1111112111211	90	0.249	
GR_3[4]	1111211111111	72	0.199	2
GR_4[4]	1112111111111	8	0.022	
GR_5	2211112111221	6	0.017	
GR_6	1111112111212	4	0.011	
GR_7	1111112111111	3	0.008	
GR_8	1111111111211	2	0.006	1
GR_9	1111112121212	2	0.006	
GR_10	1111221111111	2	0.006	2
GR_11	2211112111211	2	0.006	1
GR_12	1111111111212	1	0.003	1
GR_13	1111112211212	1	0.003	
GR_14	1111211112111	1	0.003	
GR_15	1111212111111	1	0.003	
GR_16	1121111111111	1	0.003	
GR_17	2211111111111	1	0.003	1

[1]Haplotypes are arranged in the order of decreasing frequency; [2]1 indicates the reference allele at a certain position, 2 indicates the variant allele; [3]Number of haplotypes not included in the subsequent association analysis (steroid therapy outcome, tables 4, 7 and 8) due to likelihood values ≤ 0.95; [4]Haplotype *GR_2* corresponds to haplotype b, *GR_3* corresponds to haplotype c and *GR_4* corresponds to haplotype d in Figure 2.

groups with different GC therapy outcomes, the Chi-Square test or the Fisher's exact test was used. It was analyzed whether one or two copies of a specific haplotype were associated with a particular therapy outcome compared to the GR wild-type carriers. If the number of subjects per group was large enough, heterozygous carriers with one wild-type allele and homozygous carriers of one distinct haplotype were analyzed together against homozygous wild-type carriers. The latter calculations were only performed for haplotypes which occurred in a reasonably large (> 40) number. The statistical analysis was performed using the software package SPSS 17 (SPSS Inc., Chicago, IL).

RESULTS

NR3C1 sequence variability

DNA samples from 185 IBD patients (CD or UC) were initially sequenced for the *NR3C1* coding exons 2 to 9

Table 3 Frequencies of single nucleotide polymorphisms detected in the glucocorticoid receptor gene (*NR3C1*)

SNP number	Alternative name[1]	Variant number[2]	DNA position[3]	DNA region	cDNA position[4]	Nucleotide reference	Nucleotide variant	Amino acid exchange	Allele frequency (n = 362)	Reported allele frequencies[2]
1	2.1	*rs6189*	3943266	Exon 2	558	G	A	E22E	0.025	0.002-0.034
2	2.2	*rs6190*	3943264	Exon 2	560	G	A	R23K	0.025	0.002-0.034
3	2.3	*rs72542742*	3942647	Exon 2	1177	G	A	A229T	0.003	0.002
4	2.4	*rs56149945*	3942244	Exon 2	1580	A	G	N363S	0.025	0.000-0.046
5	3.1	*rs4986593*	3856773	Intron 3		T	C		0.213	0.008-0.228
6	4.1	*rs61753484*	3852751	Intron 4		G	C		0.006	0.000-0.009
7	5.1	*rs6188*	3843271	Intron 5		G	T		0.290	0.000-0.500
8	6.1	*rs6194*	3841288	Exon 6	2256	C	T	H588H	0.006	0.000-0.091
9	8.1	*rs258751*	3825207	Exon 8	2526	C	T	D678D	0.006	0.000-0.149
10	8.2	*novel SNP*	3824968	Intron 8		A	G		0.003	NA
11	8.3	*rs258750*	3824816	Intron 8		T	C		0.307	0.091-0.362
12	8.4	*rs10482704*	3824690	Intron 8		G	T		0.017	0.000-0.027
13	9.1	*rs6196*	3824417	Exon 9	2790	T	C	N766N	0.022	0.058-0.325

[1]Defines both the exon/intron localization and the single nucleotide polymorphism (SNP) number; [2]According to the National Center for Biotechnology Information (NCBI) SNP database; [3]According to the NCBI genomic reference sequence NT_029289.11; [4]According to the NCBI cDNA reference cDNA Sequence NM_000176.2. NA: Not applicable.

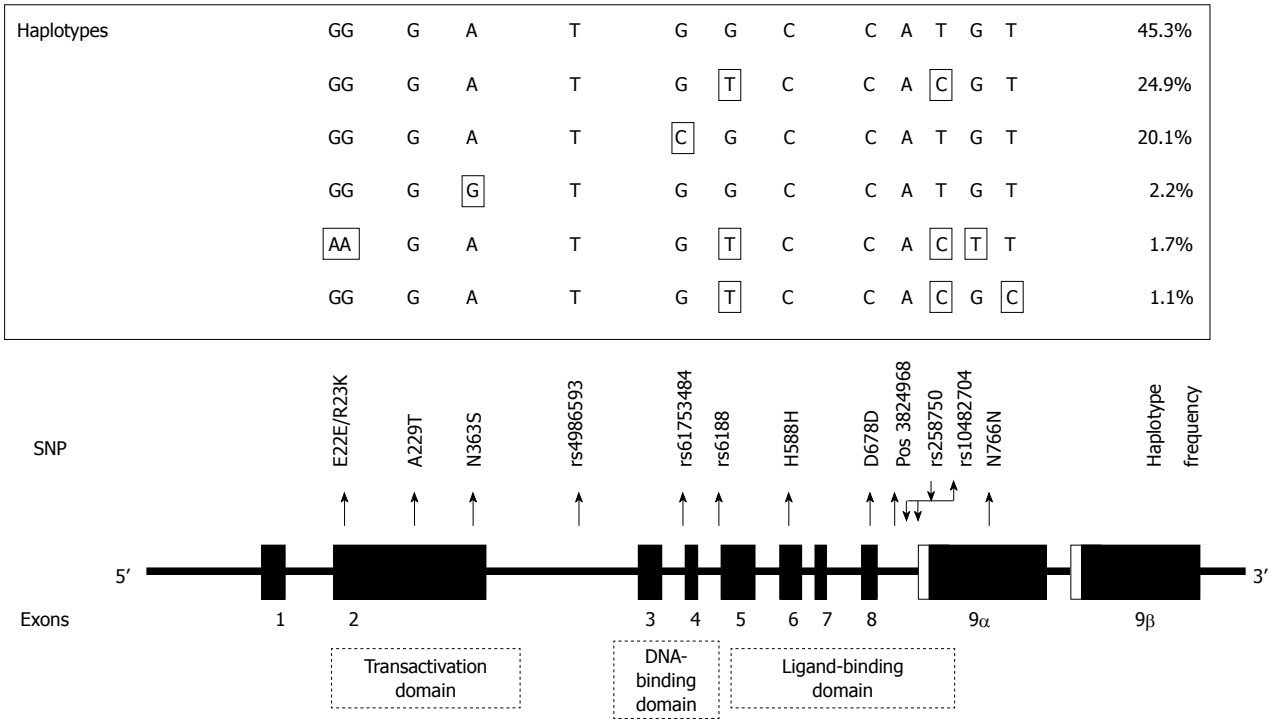

Figure 1 Most frequently occurring *NR3C1* haplotypes and their single nucleotide polymorphisms composition. The localization of the variant nucleotides in the *N3RC1* gene is indicated. All detected non-synonymous single nucleotide polymorphism (SNP) (R23K, A229T, N363S) flank the N-terminal transactivation domain. Only four out of 17 predicted haplotypes occur at a frequency higher than 2%.

and 50 bp of the neighbouring intronic sequences. The sequencing results of 181 individuals were of adequate quality and further used for SNP and haplotype analyses. The sequence data were screened for genetic variations in the *NR3C1* gene, using the Basic Local Alignment Search Tool (BLAST; www.ncbi.nih.gov) and the GenBank entry NT_029289 as the reference sequence.

In Table 3 we list the allele frequencies of all detected SNPs within the IBD cohort under study. Thirteen variants were detected, which were-with exception of one mutation (*rs6196*, $P < 0.01$)-in Hardy-Weinberg equilibrium. All variants were single nucleotide substitutions. Six variants were detected within the intronic regions, whereas seven variants were found in exons (Figure 1). Three of the seven variants detected within the coding regions of the *NR3C1* gene resulted in non-synonymous amino acid exchanges, while four of them did not lead to changes in the GR amino acid sequence. Eight variants occurred with an allelic frequency of more than one percent (*rs56149945*, *rs6189*, *rs6190*, *rs4986593*, *rs6188*, *rs258750*, *rs10482704*, *rs6196*). All non-synonymous amino acid exchanges (R23K, A229T, N363S) were found in the N-terminal half of GR, flanking the N-terminal transactivation domain[14]. The intronic variant found at

SNP		1 [2.1]	2 [2.2]	3 [2.3]	4 [2.4]	5 [3.1]	6 [4.1]	7 [5.1]	8 [6.1]	9 [8.1]	10 [8.2]	11 [8.3]	12 [8.4]	13 [9.1]	
Exon		2	2	2	4	5	6	7	8	9	10	11	12	13	
1	SNP Pos	E22E	R23K	A229T	N363S	rs4986593	rs61753484	rs6188	H588H	D678D	pos3824968	rs258750	rs10482704	N766N	Haplotype frequencies
a (wt)		G	G	G	A	T	G	G	C	C	A	T	G	T	0.4529
b		G	G	G	A	C	G	G	C	C	A	T	G	T	0.2485
c		G	G	G	A	C	G	G	C	C	A	T	G	T	0.2006
d		G	G	G	G	T	G	G	C	C	A	T	G	T	0.0219
e		A	A	G	A	T	G	T	C	C	A	C	T	T	0.0166
f		G	G	G	A	T	G	T	C	C	A	C	G	C	0.0108
g		G	G	G	A	T	G	T	C	C	A	T	G	T	0.0083
h		G	G	G	A	T	G	T	C	T	A	C	G	C	0.0055
i		G	G	G	A	T	G	G	C	C	A	C	G	T	0.0053
j		A	A	G	A	T	G	T	C	C	A	C	G	T	0.0046
k		G	G	G	A	C	C	G	C	C	A	T	G	T	0.0036
l		A	A	G	A	T	G	G	C	C	A	T	G	T	0.0035
m		G	G	G	A	C	G	T	C	C	A	T	G	T	0.0028
n		G	G		A	T	G	T	T	C	A	C	G	C	0.0028
o		G	G	A	A	T	G	G	C	C	A	T	G	T	0.0028
p		G	G	G	A	T	G	G	C	C	A	C	G	C	0.0027
q		G	G	G	A	T	C	G	C	C	A	T	G	T	0.0020
r		G	G	G	A	T	G	G	C	C	G	T	G	T	0.0016
s		G	G	G	A	C	G	G	C	C	G	T	G	T	0.0012
t		G	G	G	A	C	G	T	C	C	A	C	G	T	0.0011
u		G	G	G	A	C	G	G	C	C	A	C	G	T	0.0005
v				G	A	C	G	G	C	C	A	T	G	C	0.0002
w		A	A	G	G	T	G	G	C	C	A	T	G	T	0.0002
x		G	G	G	A	T	G	G	C	C	A	T	G	C	0.0001
y		G	G	G	G	T	G	T	C	C	A	C	G	T	0.0001

Non-synonymous coding SNP
Synonymous coding SNP
Intronic SNP

Figure 2 *NR3C1* haplotypes predicted by PHASE in the cohort of 181 inflammatory bowel diseases patients. [1]Counter (a to y) for the 25 theoretically arising haplotypes in the inflammatory bowel diseases cohort. SNP: Single nucleotide polymorphism.

DNA position 3824968 has not been previously listed in the NCBI SNP database.

Haplotype analysis

The 13 *NR3C1* variants described above were included in the haplotype calculations using the computer program PHASE. All 181 individuals were included in the haplotype prediction analysis (Figure 2). Twenty-five *NR3C1* haplotypes were predicted by PHASE to exist in the studied cohort. Furthermore, PHASE determined 17 different distinct haplotypes, which were found to be in best reconstruction for the cohort (Table 2). Six out of these 17 haplotypes occurred at a frequency higher than 1% (Figure 1). PHASE was only able to determine the haplotype structure of 174 individuals out of 181 subjects with a certainty of $\geqslant$ 95%. The data of one individual were excluded because of missing demographic data. Thus, the predicted haplotypes of 173 individuals were included in the subsequent association analysis.

The two SNPs E22E/R23K were found to be in complete linkage disequilibrium (Figure 3). This finding is in agreement with previous publications[15,16]. Furthermore, a strong but not complete linkage was found between the SNPs *rs6188* and *rs258750* (Figure 3).

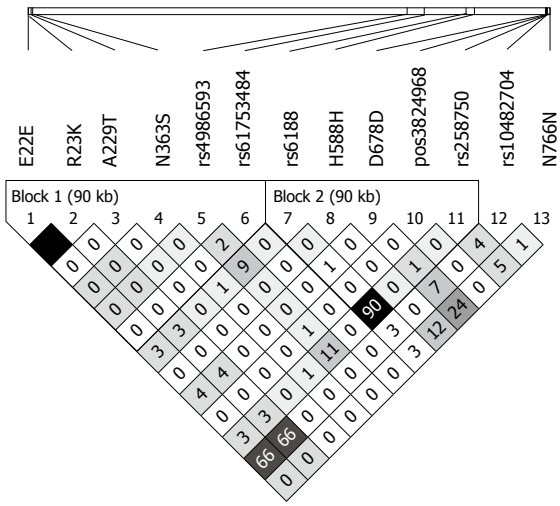

Figure 3 Linkage disequilibrium calculations of single nucleotide polymorphisms in the *NR3C1* gene. Linkage disequilibrium plot of r^2 values of observed variants in the *N3RC1* gene. Colour scheme: r^2 = 0%, white, 0% < r^2 < 100%, shades of grey, r^2 = 100%, black.

Table 4 Demographic data of 173 inflammatory bowel diseases patients included in the association analysis

Characteristics	Crohn's disease	Ulcerative colitis	All
Patients	84 (49%)	89 (51%)	173 (100%)
Age (documented for 171 individuals)	37.5 (± 15.3)	41.7 (± 14.2)	39.7 (± 14.9)
mean ± SD	35	42	39
Median	16	18	16
Minimum	72	82	82
Maximum			
Known GC treatment outcome in the past	50	50	100
No. of patients currently treated with GCs	83	60	143
Male/female	52 (58.4%)/ 37 (41.6%)	40 (47.6%)/ 44 (52.4%)	92 (53.2%)/ 81 (46.8%)
Wild-type carriers	15 (16.9%)	20 (23.8%)	35 (20.2%)
Carriers of one variant haplotype	48 (53.9%)	39 (46.4%)	87 (50.3%)
Carriers of two variant haplotypes	26 (29.2%)	25 (29.8%)	51 (29.5%)

GCs: Glucocorticoids.

Analysis of NR3C1 haplotypes in relation to steroid therapy outcome

An overview of the demographic data of the 173 subjects included in the association analysis is shown in Table 4 (further patient data on comedications and extraintestinal manifestations are given in Tables 5 and 6), and the haplotype combinations calculated for all patients are shown in Table 7. As the numbers of homozygous carriers of variant *NR3C1* haplotypes were low, the subjects were analyzed as carriers of one or two copies of a distinct variant haplotype, irrespective of whether the other allele was determined to be wild-type or variant in the case of heterozygotes (Table 8). Furthermore, haplotypes *GR_2* and *GR_3* were analyzed by testing the heterozygous allele combinations *GR_2* + *GR_1* (wt) together with the

Table 5 Past and current additional medication of 173 inflammatory bowel diseases patients included in the association analysis

Additional medication	*n*
5-Aminosalicylic acid	142
6-Mercaptopurine	33
Adalimumab	3
Antibiotics	64
Azathioprine	122
Bisphosphonates	8
Certulizumab	1
Cholestyramine	8
Cyclosporine	8
Infliximab	45
Methotrexate	27
Sulfasalazine	12
Ursodeoxycholic acid	3

Table 6 Extraintestinal manifestations

Extraintestinal manifestations	*n* (%)[1]
Peripheral arthritis	46 (27.2)
Uveitis/iritis	6 (3.6)
Pyoderma gangrenosum	4 (2.4)
Erythema nodosum	9 (5.3)
Aphthous oral ulcers	10 (5.9)
Ankylosing spondylitis	7 (4.1)
Primary sclerosing cholangitis	6 (3.6)

[1]Documented for 169 patients.

homozygous *GR_2* subjects and the allele combination *GR_3* + *GR_1* (wt) together with homozygous *GR_3* carriers against wild-type carriers. For all individuals, prior success of GC therapy was documented, and for patients under GC therapy at the point of study entry the applied dosage was also noted.

No significant associations were observed between haplotype *GR_2* and success of GC therapy (Figure 4). Upon stratification of the patient cohort according to gender or disease subgroup (UC or CD), no significant association between therapy success or haplotype *GR_2* was observed either. Similarly, when stratifying according to the subgroup of heterozygous *GR_2* + *GR_1* and homozygous *GR_2*, no statistically significant difference in therapy response compared to wild-type carriers could be observed.

No significant associations were observed between either haplotype *GR_3* (Figure 5) or *GR_4* (Table 8) and GC therapy outcome, or between individual SNPs and therapy success (Figure 6). Similarly, we observed no significant associations between the severity of disease (active or inactive state of UC or CD) or currently taken GC dose levels and *NR3C1* haplotypes (data not shown).

DISCUSSION

Glucocorticoid receptor (GR) plays an important role in many physiological and pathological processes and is the main target of glucocorticoids, widely used as therapeutic

Table 7 Predicted frequencies of haplotype combinations in 173 inflammatory bowel diseases patients

Haplotype combination	*n*	Frequency
GR_1 + *GR_2* or *GR_2* hom	51	0.295
GR_1 + *GR_3* or *GR_3* hom	41	0.237
GR_1 hom (wt)	36	0.208
GR_2 + *GR_3*	23	0.133
GR_1 + *GR_4* or *GR_4* hom	6	0.035
GR_1 + *GR_5*	3	0.017
GR_3 + *GR_6*	2	0.012
GR_1 + *GR_7* or *GR_7* hom	1	0.006
GR_1 + *GR_16* or *GR_16* hom	1	0.006
GR_2 + *GR_8*	1	0.006
GR_2 + *GR_6*	1	0.006
GR_2 + *GR_9*	1	0.006
GR_6 + *GR_13*	1	0.006
GR_7 + *GR_15*	1	0.006
GR_2 + *GR_4*	1	0.006
GR_2 + *GR_11*	1	0.006
GR_5 + *GR_9*	1	0.006
GR_9 + *GR_17*	1	0.006

Table 8 Association between glucocorticoids therapy outcome and the haplotype *GR_4*

Haplotype	Cohort composition	Therapy success rate in wt carriers (success/no success)	Therapy success rate in het/hom variant carriers (success/no success)	*P*-value	OR (CI)
GR_4 merged	All	0.682 (15/7)	0.4 (2/3)	0.326	3.214 (0.434-23.787)
	Male	0.733 (11/4)	NA (0/0)	NA	NA
	Female	0.571 (4/3)	0.4 (2/3)	1.000	2.000 (0.194-20.614)

NA: Not applicable (at least one cell box was counted as 0, OR and *P* not calculatable).

agents to treat a variety of autoimmune diseases[8,17]. Two GR isoforms, GRα and GRβ, generated by alternative mRNA splicing exist[18]. Only GRα can be activated by glucocorticoid ligands, while GRβ does not bind glucocorticoids and may in fact act as an inhibitor of glucocorticoid action[19]. Genetic variation in the *NR3C1* gene has been shown to affect both disease pathophysiology and response to glucocorticoid therapy[15,20-22], suggesting that SNPs might play a role in GR function and associated steroid therapy outcome also in IBD patients. GR is known to regulate the intestinal bile acid uptake transporter ASBT[23,24], the expression of which is altered in IBD patients[25]. While it has been reported that GR mRNA expression levels are not predictors of steroid response in IBD[26] and that the GR polymorphisms R23K and N363S are not associated with CD in a pediatric Caucasian population[27], no studies on the role of *NR3C1* gene variants in steroid therapy success were previously available. The aim of the current study was to analyze sequence variation and

Haplotype	Cohort composition	Therapy success rate in wt carriers (No. of success/no success)	Therapy success rate in het/hom variant carriers (No. of success/no success)	*P*-value	OR (CI)
GR_2 merged	All	0.682 (15/7)	0.837 (36/7)	0.204	0.417 (0.124-1.395)
	Male	0.733 (11/4)	0.905 (19/2)	0.210	0.289 (0.045-1.846)
	Female	0.571 (4/3)	0.773 (17/5)	0.357	0.392 (0.065-2.369)
	CD	0.615 (8/5)	0.900 (18/2)	0.084	0.178 (0.028-1.118)
	UC	0.778 (7/2)	0.783 (18/5)	1.000	0.972 (0.152-6.231)
GR_2 only	All	0.682 (15/7)	0.862 (25/4)	0.173	0.343 (0.086-1.370)
	Male	0.733 (11/4)	0.875 (14/2)	0.394	0.393 (0.060-2.554)
	Female	0.571 (4/3)	0.846 (11/2)	0.290	0.242 (0.029-2.027)

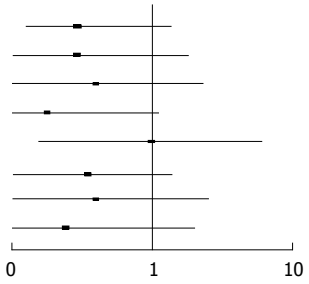

Figure 4 Haplotype *GR_2* and steroid therapy outcome. Odds ratios and confidence intervals for the number of *GR_2* carriers *vs* wild-type carriers in the responder group compared with non-responders to glucocorticoid therapy. No significant associations were found. Statistical analysis was performed with Fisher's exact test. OR: Odds ratio; CI: Confidence interval; CD: Crohn's disease; UC: Ulcerative colitis.

Haplotype	Cohort composition	Therapy success rate in wt carriers (No. of success/no success)	Therapy success rate in het/hom variant carriers (No. of success/no success)	*P*-value	OR (CI)
GR_2 merged	All	0.682 (15/7)	0.318 (28/5)	0.188	0.383 (0.103-1.415)
	Male	0.733 (11/4)	0.913 (4/1)	0.342	0.250 (0.024-2.609)
	Female	0.571 (4/3)	0.810 (17/4)	0.318	0.314 (0.049-1.998)
	CD	0.615 (8/5)	0.917 (11/1)	0.160	0.145 (0.014-1.489)
	UC	0.778 (7/2)	0.810 (17/4)	1.000	0.824 (0.122-5.573)
GR_2 only	All	0.682 (15/7)	0.842 (16/3)	0.292	0.402 (0.087-1.846)
	Male	0.733 (11/4)	1.000 (6/0)	0.281	NA
	Female	0.571 (4/3)	0.769 (10/3)	0.613	0.400 (0.055-2.886)

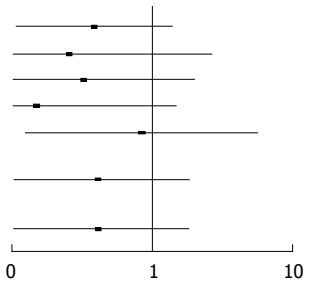

Figure 5 Haplotype *GR_3* and steroid therapy outcome. Odds ratios and confidence intervals for the number of *GR_3* carriers *vs* wild-type carriers in the responder group of responders compared with non-responders to glucocorticoid therapy. No significant associations were found. Statistical analysis was performed using Fisher's exact test. CD: Crohn's disease; UC: Ulcerative colitis; NA: Not applicable.

Mutation	Therapy success rate in wt carriers (success/no success)	Therapy success rate in het/hom mutated carriers (success/no success)	*P*-value	OR (CI)
E22E/R23K	0.774 (72/21)	0.571 (4/3)	0.354	2.571 (0.533-12.408)
N363S	0.777 (73/21)	0.500 (3/3)	0.148	3.476 (0.653-18.508)
rs4986593	0.746 (47/16)	0.784 (29/8)	0.670	0.810 (0.308-2.131)
rs6188	0.714 (35/14)	0.804 (41/10)	0.294	0.610 (0.241-1.543)
rs258750	0.694 (34/15)	0.824 (42/9)	0.129	0.486 (0.189-1.246)
rs10482704	0.760 (73/23)	0.750 (3/1)	1.000	1.058 (0.105-10.671)

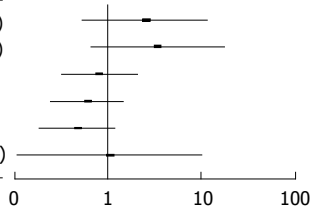

Figure 6 *NR3C1* variants and their influence on steroid therapy outcome. Odds ratios and confidence intervals for carriers of six single nucleotide polymorphisms against wild-type carriers in the group of glucocorticoid (GC) responders compared with GC non-responders. No significant associations were found. Statistical analysis was performed using chi-square or Fisher's exact test.

haplotype structures in the coding parts of the *NR3C1* gene in a cohort of 181 Swiss IBD patients. We investigated whether *NR3C1* genetic variants or haplotypes may influence steroid therapy outcome in IBD patients.

We identified 13 variants in this study, of which 12 had already been previously submitted to the NCBI SNP database. We calculated the corresponding haplotypes in the IBD patient cohort and studied the association of the most prevalent SNP combinations with steroid therapy outcome, disease activity, and age of disease onset. Several *NR3C1* SNPs have been previously associated with altered disease susceptibility or risk of disease progression in other autoimmune diseases, such as Guillain-Barré Syndrome or multiple sclerosis[15,20]. Most of these studies only analyzed the impact of a small number of pre-defined SNPs, such as the BclI polymorphism or the E22E/R23K polymorphisms[15,20,21]. Few reports have been published on the potential influence of *NR3C1* SNPs on sensitivity to endogenous or exogenously given GCs[17,28], and only one significant association between the polymorphism E22E/R23K and sensitivity to exogenously administered GCs in elderly Dutch people has been reported[22]. So far no large cohort studies have been reported in which the influence of *NR3C1* SNPs on GC therapy outcome in IBD patients

has been investigated. Here, we describe five *NR3C1* haplotypes occurring at a frequency > 1% and analyze the potential association of the three most common haplotypes *GR_2*, *GR_3* and *GR_4* with GC therapy outcome in IBD patients. While a large number of *NR3C1* variants are already registered in the NCBI SNP database, we observed only eight variants that occurred at a frequency > 1%, and these were responsible for the composition of a relatively small group of commonly occuring haplotypes. The overall risk for a certain UC and/or CD activity state or for a different steroid therapy outcome was not altered in *GR_2*, *GR_3* or *GR_4* carriers, in comparison with the wild-type carriers. Furthermore, no significant associations were observed between individual SNPs and GC therapy success. In the case of certain SNPs/haplotypes (e.g. *GR_4*, E22E/R23K), a larger cohort would have been preferable in order to obtain more reliable results, as these variants occurred quite rarely in our patient group. Similarly to our observations, Dekker *et al*[20] could not detect any associations between distinct haplotypes and SNPs in a Guillain-Barré Syndrome cohort treated with methylprednisolone, although the authors noted that their study group was too small to obtain statistically reliable results. It remains to be seen whether the rare GR variants present in our study cohort will show significant associations in larger cohorts of IBD patients.

In conclusion, we have performed a comprehensive study analyzing the role of genetic variants in the *NR3C1* gene in glucocorticoid sensitivity in a Swiss cohort of IBD patients. We show that *NR3C1* haplotypes are not a general modulating factor in glucocorticoid therapy outcome.

ACKNOWLEDGMENTS

We thank Christian Hiller for excellent technical assistance, members of our team for fruitful discussions, and the Swiss IBD Cohort Study Group: Michetti P, Vader JP, Burnand B, Pittet V, Ballabeni P, Mueller C, Rihs S, Braegger C, Mottet C, Juillerat P, Felley C, Beglinger C, Froehlich F, Vavricka S, Seibold F, Schoepfer A, von Kaenel R, Begré S, de Saussure P, Meyenberger C, Sagmeister M, Rogler G, Kullak-Ublick GA, Meier R, Straumann A, Criblez D, Engelmann M and Schenk M.

COMMENTS

Background

Crohn's disease and ulcerative colitis are two distinct types of inflammatory bowel disease (IBD), which is an increasingly prevalent disease condition worldwide. Wide variation is observed in clinical manifestation and therapy responses in IBD, partly due to individual genetic variation.

Research frontiers

Glucocorticoid therapy is commonly used in treatment of IBD, however the response to therapy varies between individuals. The authors hypothesized that genetic variation in the *NR3C1* gene encoding the glucocorticoid receptor (GR) may affect the response to glucocorticoids in IBD patients.

Innovations and breakthroughs

In this comprehensive genetic analysis, all coding exons and exon-intron junctions of the *NR3C1* gene were sequenced in 181 IBD patients, who had been treated with glucocorticoids and whose past responses to this treatment had been recorded. This is the first published study on the effects of genetic variation in GR on glucocorticoid therapy in IBD patients, in a modestly sized study cohort.

Applications

If significant associations between genetic GR variants and glucocorticoid therapy outcome had been observed, this could have allowed more considered design of the individual therapy options upon prior genotyping of the patients.

Terminology

The transcription factor of the steroid receptor family, GR, is proposed to be a major mediator of anti-inflammatory pathways elicited by therapeutically administered glucocorticoids.

Peer review

The genetic study investigates the predictive value of *NR3C1* gene variants towards the clinical outcome of patients with Crohn's disease and ulcerative colitis. Although the result of this study was negative, the study was meaningful in that abundant GR variants were determined and analyzed in IBD patients.

REFERENCES

1 **Ferguson LR**, Huebner C, Petermann I, Gearry RB, Barclay ML, Demmers P, McCulloch A, Han DY. Single nucleotide polymorphism in the tumor necrosis factor-alpha gene affects inflammatory bowel diseases risk. *World J Gastroenterol* 2008; **14**: 4652-4661

2 **Glas J**, Stallhofer J, Ripke S, Wetzke M, Pfennig S, Klein W, Epplen JT, Griga T, Schiemann U, Lacher M, Koletzko S, Folwaczny M, Lohse P, Göke B, Ochsenkühn T, Müller-Myhsok B, Brand S. Novel genetic risk markers for ulcerative colitis in the IL2/IL21 region are in epistasis with IL23R and suggest a common genetic background for ulcerative colitis and celiac disease. *Am J Gastroenterol* 2009; **104**: 1737-1744

3 **Weersma RK**, Stokkers PC, van Bodegraven AA, van Hogezand RA, Verspaget HW, de Jong DJ, van der Woude CJ, Oldenburg B, Linskens RK, Festen EA, van der Steege G, Hommes DW, Crusius JB, Wijmenga C, Nolte IM, Dijkstra G. Molecular prediction of disease risk and severity in a large Dutch Crohn's disease cohort. *Gut* 2009; **58**: 388-395

4 **Pierik M**, Rutgeerts P, Vlietinck R, Vermeire S. Pharmacogenetics in inflammatory bowel disease. *World J Gastroenterol* 2006; **12**: 3657-3667

5 **Faubion WA Jr**, Loftus EV Jr, Harmsen WS, Zinsmeister AR, Sandborn WJ. The natural history of corticosteroid therapy for inflammatory bowel disease: a population-based study. *Gastroenterology* 2001; **121**: 255-260

6 **Munkholm P**, Langholz E, Davidsen M, Binder V. Frequency of glucocorticoid resistance and dependency in Crohn›s disease. *Gut* 1994; **35**: 360-362

7 **Farrell RJ**, Kelleher D. Glucocorticoid resistance in inflammatory bowel disease. *J Endocrinol* 2003; **178**: 339-346

8 **Russcher H**, Smit P, van den Akker EL, van Rossum EF, Brinkmann AO, de Jong FH, Lamberts SW, Koper JW. Two polymorphisms in the glucocorticoid receptor gene directly affect glucocorticoid-regulated gene expression. *J Clin Endocrinol Metab* 2005; **90**: 5804-5810

9 **van Winsen LL**, Hooper-van Veen T, van Rossum EF, Polman CH, van den Berg TK, Koper JW, Uitdehaag BM. The impact of glucocorticoid receptor gene polymorphisms on glucocorticoid sensitivity is outweighted in patients with multiple sclerosis. *J Neuroimmunol* 2005; **167**: 150-156

10 **van Rossum EF**, Koper JW, Huizenga NA, Uitterlinden AG, Janssen JA, Brinkmann AO, Grobbee DE, de Jong FH, van Duyn CM, Pols HA, Lamberts SW. A polymorphism in the glucocorticoid receptor gene, which decreases sensitivity to glucocorticoids in vivo, is associated with low insulin and cholesterol levels. *Diabetes* 2002; **51**: 3128-3134

11 **Pittet V**, Juillerat P, Mottet C, Felley C, Ballabeni P, Burnand B, Michetti P, Vader JP. Cohort profile: the Swiss Inflammatory Bowel Disease Cohort Study (SIBDCS). *Int J Epidemiol* 2009; **38**: 922-931

12 **Stephens M**, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001; **68**: 978-989

13 **Stephens M**, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* 2003; **73**: 1162-1169

14 **Giguère V**, Hollenberg SM, Rosenfeld MG, Evans RM. Functional domains of the human glucocorticoid receptor. *Cell* 1986; **46**: 645-652

15 **van Winsen LM**, Hooper-van Veen T, van Rossum EF, Koper JW, Barkhof F, Polman CH, Uitdehaag BM. Glucocorticoid receptor gene polymorphisms associated with more aggressive disease phenotype in MS. *J Neuroimmunol* 2007; **186**: 150-155

16 **Koper JW**, Stolk RP, de Lange P, Huizenga NA, Molijn GJ, Pols HA, Grobbee DE, Karl M, de Jong FH, Brinkmann AO, Lamberts SW. Lack of association between five polymorphisms in the human glucocorticoid receptor gene and glucocorticoid resistance. *Hum Genet* 1997; **99**: 663-668

17 **Russcher H**, Smit P, van Rossum EF, van den Akker EL, Brinkmann AO, de Heide LJ, de Jong FH, Koper JW, Lamberts SW. Strategies for the characterization of disorders in cortisol sensitivity. *J Clin Endocrinol Metab* 2006; **91**: 694-701

18 **Hollenberg SM**, Weinberger C, Ong ES, Cerelli G, Oro A, Lebo R, Thompson EB, Rosenfeld MG, Evans RM. Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature* 1985; **318**: 635-641

19 **Bamberger CM**, Bamberger AM, de Castro M, Chrousos GP. Glucocorticoid receptor beta, a potential endogenous inhibitor of glucocorticoid action in humans. *J Clin Invest* 1995; **95**: 2435-2441

20 **Dekker MJ**, van den Akker EL, Koper JW, Manenschijn L, Geleijns K, Ruts L, van Rijs W, Tio-Gillen AP, van Doorn PA, Lamberts SW, Jacobs BC. Effect of glucocorticoid receptor gene polymorphisms in Guillain-Barré syndrome. *J Peripher Nerv Syst* 2009; **14**: 75-83

21 **van Winsen LM**, Manenschijn L, van Rossum EF, Crusius JB, Koper JW, Polman CH, Uitdehaag BM. A glucocorticoid receptor gene haplotype (TthIII1/ER22/23EK/9beta) is associated with a more aggressive disease course in multiple sclerosis. *J Clin Endocrinol Metab* 2009; **94**: 2110-2114

22 **van Rossum EF**, Koper JW, van den Beld AW, Uitterlinden AG, Arp P, Ester W, Janssen JA, Brinkmann AO, de Jong FH, Grobbee DE, Pols HA, Lamberts SW. Identification of the BclI polymorphism in the glucocorticoid receptor gene: association with sensitivity to glucocorticoids in vivo and body mass index. *Clin Endocrinol* (Oxf) 2003; **59**: 585-592

23 **Jung D**, Fantin AC, Scheurer U, Fried M, Kullak-Ublick GA. Human ileal bile acid transporter gene ASBT (SLC10A2) is transactivated by the glucocorticoid receptor. *Gut* 2004; **53**: 78-84

24 **Eloranta JJ**, Jung D, Kullak-Ublick GA. The human Na + -taurocholate cotransporting polypeptide gene is activated by glucocorticoid receptor and peroxisome proliferator-activated receptor-gamma coactivator-1alpha, and suppressed by bile acids *via* a small heterodimer partner-dependent mechanism. *Mol Endocrinol* 2006; **20**: 65-79

25 **Wojtal KA**, Eloranta JJ, Hruz P, Gutmann H, Drewe J, Staumann A, Beglinger C, Fried M, Kullak-Ublick GA, Vavricka SR. Changes in mRNA expression levels of solute carrier transporters in inflammatory bowel disease patients. *Drug Metab Dispos* 2009; **37**: 1871-1877

26 **Hausmann M**, Herfarth H, Schölmerich J, Rogler G. Glucocorticoid receptor isoform expression does not predict steroid treatment response in IBD. *Gut* 2007; **56**: 1328-1329

27 **Sanchez R**, Levy E, Costea F, Sinnett D. IL-10 and TNF-alpha promoter haplotypes are associated with childhood Crohn's disease location. *World J Gastroenterol* 2009; **15**: 3776-3782

28 **van Rossum EF**, Russcher H, Lamberts SW. Genetic polymorphisms and multifactorial diseases: facts and fallacies revealed by the glucocorticoid receptor gene. *Trends Endocrinol Metab* 2005; **16**: 445-450

S- Editor Wang JL **L- Editor** O'Neill M **E- Editor** Ma WH